Pflugers Arch - Eur J Physiol (2004) 447:744–751
DOI 10.1007/s00424-003-1070-7

The ABC of Solute Carriers

Guest Editor: Matthias A. Hediger

Richard D. Palmiter · Liping Huang

Efflux and compartmentalization of zinc by members of the SLC30 family of solute carriers

Received: 10 January 2003 / Accepted: 21 March 2003 / Published online: 14 May 2003

Abstract All of the members of this family are thought to facilitate zinc efflux from the cytoplasm either into various intracellular compartments (endosomes, secretory granules, synaptic vesicles, Golgi apparatus, or trans-Golgi network) or across the plasma membrane. Thus, these transporters are thought to help maintain zinc homeostasis and facilitate transport of zinc into specialized intracellular compartments. Counterparts of the SLC30 family are found in all organisms. Most of the members of this class are predicted to have 6 transmembrane domains with both N- and C-termini on the cytoplasmic side of the membrane. Expression of rodent *Znt1, Znt2* or *Znt4* cDNAs in mammalian cells can confer resistance to zinc toxicity. Loss of function of the mouse *Znt1* is embryonic lethal, loss of mouse *Znt3* prevents accumulation of zinc in synaptic vesicles, nonfunctional mouse *Znt4* (*lethal milk*) results in zinc-deficient milk, and *Znt5*-null mice display bone abnormalities and heart failure. No mutations in human counterparts of any of the members of the SLC30 family have been described.

History

Baby hamster kidney (BHK) cells that had been transfected with a zinc-responsive reporter gene (*MRE-βGeo*) were extensively mutagenized, and screened for variants with high basal expression of the reporter gene. One recessive clone was identified that not only had a high basal expression of the reporter gene but was also more sensitive to zinc toxicity [1]. This afforded an easy means to select for genes that could confer resistance to zinc toxicity. Rat *Znt1* and *Znt2* cDNAs were recovered from this screen [1, 2]; sequencing revealed that they were homologous to yeast genes *ZRC1* and *COT1*, which had been shown to confer resistance to zinc toxicity [3, 4]. *Znt3* was cloned by low-stringency hybridization of a mouse genomic library with a rat *Znt2* cDNA probe [5]. *Znt4* was cloned serendipitously during a search for the *pallid* gene on mouse chromosome 2 [6]; a nonsense mutation in *Znt4* is responsible for the *lethal milk* mouse that arose spontaneously at the Jackson laboratory [7, 8]. Mouse *Znt5* and human *ZNT5*, *Znt6 (ZNT6)* as well as *Znt7 (ZNT7)* were identified by searching through DNA databases with sequences derived from other members of the SLC30 family [9, 10, 11]. Human *ZNT*8 and *ZNT9* were recognized from recent database analysis but no experimental analysis has been published. Over 100 members of the SLC30 family are recognized in organisms ranging from prokaryotes, *Caenorhabditis elegans, Drosophila melanogaster* to plants, indicating that this is an ancient family [12], which is not surprising because regulating cytoplasmic zinc concentration is essential for homeostasis.

R. D. Palmiter (✉)
Howard Hughes Medical Institute and Department of Biochemistry, University of Washington,
Box 357370, Seattle, WA 98195, USA
e-mail: Palmiter@u.washington.edu
Tel.: +1-206-5436064, Fax: +1-206-5430858

L. Huang
Western Human Nutrition Research Center/Agricultural Research Service/United States Department of Agriculture,
Department of Nutrition and the Rowe Program in Genetics, University of California, Davis, CA 95616, USA

General characteristics of SLC30 family members

The SLC30 family was previously called the cation diffusion facilitator (CDF) family [12, 13, 14]. Nine members of this family are recognized in mammals (Table 1). Based on sequence similarity, Gaither and Eide [12] divided the CDF family into three subfamilies. An orphan, *ZNT9* resides in subfamily I, while *ZNT1, 5, 6, 7, 8* fall into subfamily II, and the others (*ZNT2, 3, 4*) are in subfamily III (Fig. 1). All of the members of the SLC30 family are predicted to have six transmembrane domains with both N- and C-termini on the cytoplasmic side of the membrane (as shown in Fig. 2) with the exception of *ZNT5*, which has a large N-terminal extension with an

Table 1 SLC30: the zinc efflux family

Human gene name	Protein name	Aliases	Prominent substrate	Transport type	Distribution	Link to disease	Human gene locus	Sequence accession number
SLC30A1	ZNT1		Zinc	Unknown	Widespread[a]	Embryonic lethal[b]	1q32.3	NM_021194
SLC30A2	ZNT2		Zinc	Unknown	Widespread[a]		1p35.3	NM_032513
SLC30A3	ZNT3		Zinc	Unknown	Glutamatergic neurons	Seizures, Alzheimer's[b]	2p23.3	NM_003459
SLC30A4	ZNT4	Dri27	Zinc	Unknown	Widespread[a] secretory glands	*Lethal milk* [b]	15q21.1	NM_013309
SLC30A5	ZNT5	ZTL1	Zinc[d]	Unknown	Widespread; β cells	Bone abnormalities; heart failure[b]	5q13.1	NM_022902
SLC30A6	ZNT6		Zinc[d]	Unknown	Widespread[A]		2p22.3	NM_017964
SLC30A7	ZNT7	ZNTL2	Zinc	Unknown	Widespread[a]		1p21.2	NM_133496
SLC30A8	ZNT8		Unknown	Unknown	Brain, liver		1q41	AY212919
SLC30A9[c]	ZNT9[c]	C4orf1	Unknown	Unknown	Unknown		4p13	NM_006345

[a] By RNA analysis in rodents [b] In mouse [c] Orphan transporter
[d] By association only, no zinc-dependent phenotypes described in mammalian cells

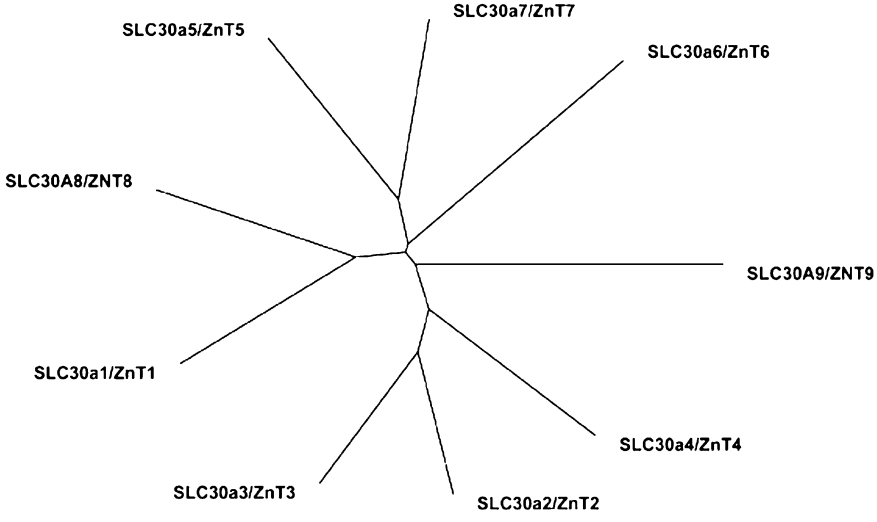

Fig. 1 Tree view of the relationships among SLC30 family members. Amino acid sequences of mouse ZnT1–7 and human ZNT8 and 9 were used to generate the tree. ZnT1 has 38.4% identity with ZNT8. ZnT2 has 53.4% identity with ZnT3, and 42.4% identity with ZnT4. ZnT5 has 25.2% identity with ZnT6, and 45.2% identity with ZnT7. The percentage identities were obtained with Genetics Computer Group Gap Program

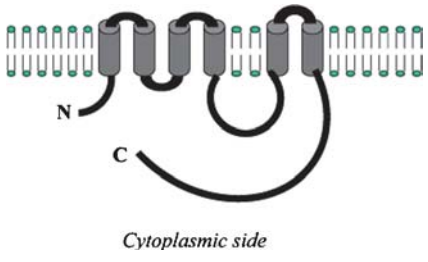

Fig. 2 Predicted topology of SLC30 class of zinc efflux transporters. Most members have 6 transmembrane domains; however, ZnT5 has 6–9 additional transmembrane segments at the N-terminus. ZnT2 is the smallest (359 aa), while ZnT5 is the largest (765 aa)

estimated six to nine additional transmembrane domains. Most of the proteins have a long C-terminal tail. They tend to have small loops connecting the transmembrane domains except for the intracellular loop between domains IV and V, which is usually rich in histidine residues. They are all thought to transport zinc from the cytoplasm either out of the cell or into intracellular compartments (Fig. 3). *ZNT1* has the simplest gene structure (only 2 exons). Little is known about the energetics of zinc transport, cofactors or coupling ions for any members of the mammalian SLC30 family [1]. Studies on the *Bacillus subtilis* CDF protein, CzcD, indicate that it is an antiporter, exchanging Zn^{2+} for H^+ or K^+, while the yeast CDF protein, ZRC1, which facilitates Zn^{2+} transport into the vacuole, depends on the proton gradient [15, 16]. Zinc is the only metal known to be transported by members of the mammalian SLC30 family under physiological conditions.

Zinc homeostasis by cells depends on transport of zinc in both directions across the plasma membrane as well as both in and out of various vesicular compartments. The SLC39 family includes the ZIP proteins that facilitate zinc transport in the opposite direction to the SLC30 family members. For example, human ZIP1 and ZIP2 proteins facilitate zinc uptake in mammalian cells countering zinc efflux by ZNT1. Likewise, yeast ZRC1 and COT1 facilitate zinc uptake into the vacuole while ZRT3

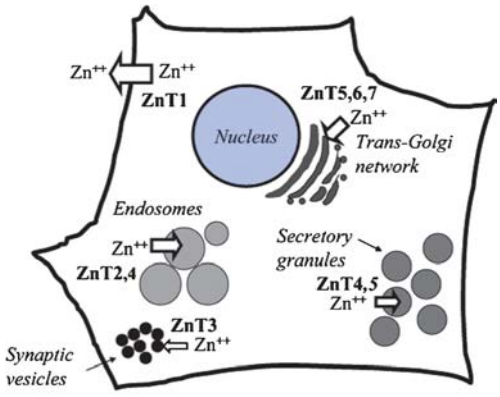

Fig. 3 Cellular localization of SLC30 class of zinc efflux transporters. ZnT1 is targeted to plasma membrane where it facilitates zinc efflux from cells; all the other members facilitate zinc transport into various intracellular compartments. Some of the transporters have been found in different compartments in different cells (see text). All of family members presumably transit from endoplasmic reticulum, through the trans-Golgi network during biosynthesis. Overexpression of these proteins in heterologous cells may result in inappropriate cellular trafficking if in the absence of appropriate chaperones

facilitates its efflux, thereby allowing the vacuole to serve as a reservoir for zinc storage [12]. It is likely that members of the mammalian ZIP family will facilitate zinc efflux from endoplasmic reticulum, Golgi, endosomes, and secretory compartments.

In multicellular organisms, metal ion transport from the environment (food) into the circulatory system relies upon transport across epithelial cells lining the gut. The DCT1/DMT1/Nramp2 protein (a member of the SLC11 family) has been implicated in uptake of zinc and other divalent metals [17]. Utilization of zinc in the diet depends upon uptake at the apical surface of epithelial cells and subsequent efflux at the basal surfaces. The same principle applies to placental transport of zinc to the fetus from the mother during mammalian development. Consequently, defects in zinc availability to a fetus or an adult could reflect defects in either zinc uptake at the apical surface or zinc efflux at the basal surface of epithelial cells. Mammals can tolerate very high concentrations of zinc in their drinking water, e.g., mice can be maintained for months on a diet containing 25 mM zinc sulfate in their water, whereas cultured cells fail to thrive when zinc levels exceed ~0.2 mM. Because epithelial cells lining the gut provide the first line of defense against absorption of excessive zinc, they undoubtedly have many protective mechanisms including regulation of zinc transport and sequestration of zinc.

Pharmaceutical aspects of the SLC30 family

We are unaware of any compounds that target this class of transporters or of any efforts to search for them.

SLC30A1 (human ZNT1; rodent Znt1)

Tissue distribution

Tissue distribution. Most of the available information is derived from studies in rodents. By RT-PCR and Northern blot analysis, Znt1 is expressed in all tissues examined [5, 18, 19], expression of mRNA and protein has been studied in liver, intestine [18] and brain [20]. Expression in embryonic stem cells is implied (see Functional studies).

Cellular localization

ZnT1 is the only member of this family located primarily on the plasma membrane. Transfection of BHK cells with constructs allowing expression of ZnT1 with EGFP fused to either the N- or C-terminus manifests abundant localization on the plasma membrane and these constructs retain function (unpublished observations).

Regulation of expression

Znt1 gene expression is regulated by zinc. Increases in dietary zinc induced intestinal, but not hepatic, *Znt1* mRNA levels [18, 19] and zinc deficiency lowers *Znt1* mRNA in visceral yolk sac [21]. Forebrain ischemia, which probably releases zinc into the extracellular space, induces *Znt1* expression within the hippocampus of gerbils [22]. The transcription factor, MTF-1, which responds to changes in intracellular zinc and mediates metallothionein gene induction, is implicated in regulation of *Znt1* [21]. However, genomic DNA containing the *Znt1* gene can confer zinc resistance to cells lacking MTF-1, indicating that this transcription factor is not essential for basal levels of expression (unpublished observations). The *Znt1* gene was inactivated in mouse embryonic stem cells by targeting the *βGeo* gene (a *lacZ-neomycin resistance* gene fusion) to the *Znt1* locus such that *βGeo* would be under control of *Znt1* gene regulatory elements. Although this gene confers resistance to G418, *β*-galactosidase activity is barely detectable and there is minimal regulation by zinc (unpublished observations).

Functional studies

The rat *Znt1* cDNA was discovered by virtue of its ability to confer zinc resistance to a zinc-sensitive BHK cell line when expressed from a CMV promoter [1]. Transfection of genomic DNA containing the mouse *Znt1* gene can also confer resistance to zinc toxicity [1]. Gradual selection for cells that can grow at higher and higher concentrations of zinc results in amplification (~tenfold) of the endogenous *Znt1* gene. Amplification has been obtained in both "wild-type" BHK cells and mouse cells lacking MTF-1, but similar selection starting with the

zinc-sensitive BHK cells failed (unpublished observations). After months of selection, the zinc-resistant cells could grow at concentrations (0.5 mM) that kill all parental cells. As expected, the rate of ^{65}Zn efflux was greatly accelerated and the dose-response for induction of a zinc-responsive reporter gene was shifted to higher concentrations of zinc (unpublished observations). All of these results are consistent with the idea that ZnT1 confers resistance to zinc by increased efflux of zinc across the plasma membrane.

The zinc-sensitive BHK cells that were obtained by mutagenesis probably lack functional ZnT1, although the genetic defect has not been established. This conclusion is supported by the observation that *Znt1* gene amplification has not been observed in this cell line and dominant-negative constructs (see below) have no further effect in this cell line [1]. The *Znt1* gene was inactivated in ES cells by gene targeting (see above). Inactivation of both *Znt1* alleles was achieved in ES cells by selection in high concentrations of G418. These cells grow normally and show minimal increase in sensitivity to zinc toxicity. *Znt1* heterozygous mice were generated and appear to be normal, but all *Znt1* homozygotes die by embryonic day 10 (unpublished observations). Thus, ZnT1 function is essential for survival. However, it is not clear whether ZnT1 is required to protect embryos from zinc toxicity, or more likely, to make zinc available to the embryo by facilitating zinc efflux from placental tissues.

Functional analyses of a few truncations of ZnT1 have been performed. An N-terminal deletion that removes the first transmembrane domain results in a protein that has dominant-negative effects on zinc efflux [1]. Deletions of the C-terminal cytoplasmic tail results in a protein that is toxic to BHK cells [1]. More extensive functional analysis of ZnT1 would be relatively easy because one can select directly for activity using the zinc-sensitive BHK cell line and ^{65}Zn-efflux assays are easy [1].

Physiological implications

ZnT1 is essential for survival of the mouse embryo; thus, conditional inactivation will be essential to explore its roles in adult tissues. ZnT1 was discovered by virtue of its ability to confer resistance to zinc toxicity and most cell culture studies continue to exploit this property. This role is most easily demonstrated in rare cell lines that cannot make metallothionein because these proteins also confer resistance to zinc toxicity. ZnT1 may provide protection against zinc toxicity in animals under conditions where large amounts of zinc encountered suddenly, e.g., in the gut and in brain in response to ischemia and seizures (see section SLC30A3). However, we suspect that a more common function will be to facilitate export of zinc from one compartment to another, e.g., from intestinal cells to the bloodstream or from exocrine cells to the pancreatic ducts.

SLC30A2 (human ZNT2; rodent Znt2)

Tissue distribution

Znt2 mRNA was detected by RT-PCR in most tissues examined [2]. By Northern blot, *Znt2* mRNA was found in small intestine, kidney, placenta and sometimes in liver [18].

Cellular localization

Rat *Znt2* cDNA was discovered by virtue of its ability to confer resistance to zinc toxicity in a zinc-sensitive BHK cell line [2]. Unlike *Znt1*, which was discovered in the same screen, expression of *Znt2* leads to the accumulation of zinc in intracellular organelles that have the characteristics of late endosomes [2, 23]. The zinc in this compartment can be easily visualized with compounds such as zinquin, which fluoresce in the presence of zinc. Exposure to high concentrations of zinc leads to accumulation of zinc-laden endosomes and the zinc escapes from those vesicles very slowly, if at all [2]. Although ZnT2 protein is localized to late endosomes in the transfected BHK cells, this may not be its normal intracellular location because appropriate intracellular targeting may depend on chaperones that are not present in BHK cells (see sections SLC30A3 and SLC30A4).

Regulation of expression

Znt2 mRNA is induced robustly in small intestine, liver and kidney in response to large oral dose of zinc [19].

Functional studies

Expression of ZnT2 confers resistance to zinc in BHK cells that lack the ability to make metallothioneins, whereas it is difficult to demonstrate this function in cells that can express these zinc-binding proteins [2]. No structure/function analysis of ZnT2 has been reported. Rat ZnT2 protein initiates with a CTG codon rather than the consensus ATG [2].

Physiological implications

The fact that *Znt2* mRNA is robustly induced by zinc in vivo suggests that it may play a role in protecting against transient rises in zinc.

SLC30A3 (human ZNT3; rodent Znt3)

Tissue distribution

Znt3 mRNA is most abundant in brain and testis; however, the ZnT3 protein was undetectable in testis [5].

Cellular localization

ZnT3 protein is localized to synaptic vesicles of glutamatergic neurons that sequester zinc [24]. The amount of ZnT3 protein detected by immunocytochemistry correlates well with the amount of vesicular zinc that can be detected by either the Timm's stain or by fluorescent dyes such a zinquin or TSQ [24, 25]. The *mocha* mutation reduces ZnT3 protein and vesicular zinc, suggesting that the AP3 chaperone complex is necessary for the normal trafficking of ZnT3 to synaptic vesicles [26].

Regulation of expression

There is little information on the regulation of *Znt3* gene expression. *Znt3* mRNA is detectable in brain a few days before birth and reaches adult levels by 3 weeks postpartum [25].

Functional studies

The *Znt3* gene was inactivated in embryonic stem cells by targeting the *lacZ* gene to the locus. Mice heterozygous for *Znt3* are normal but have half the normal amount of vesicular zinc in the brain [25]. *Znt3*-null mice completely lack vesicular zinc in neurons [25] but they appear normal. They reproduce normally and no behavioral or learning disabilities have been revealed [25, 27]. The *Znt3*-null mice are more sensitive to kainite-induced seizures, but surprisingly had similar sensitivity to other proconvusant agents—bicuculline, pentylenetetrazol and flurothyl [28]. Despite the lack of synaptic vesicle zinc that can be released in response to seizures, zinc still accumulates in post-synaptic cells after seizures and there is still extensive neuronal damage in mice lacking Znt3 [28, 29]. Electrophysiological properties of hippocampal slices from control and *Znt3*-null mice are remarkably normal [30]. Zinc contributes to the aggregation of amyloid peptides in Alzheimer's disease [31]. Transgenic mice expressing a mutant human amyloid precursor protein (hAPP) develop amyloid plaques after 12 months of age. Inactivation of *Znt3* alleles in the transgenic *hAPP* background greatly reduced the appearance of amyloid plaques, suggesting that the release of zinc from synaptic vesicles contributes to amyloid deposition in this model [32].

Expression of *Znt3* cDNA in BHK cells has no effect on zinc resistance and no vesicular pool of zinc can be visualized, perhaps because appropriate molecular chaperones, such as AP3, are required (see section Cellular localization).

Physiological implications

Because zinc is sequestered in synaptic vesicles along with glutamate and these molecules are co-released, the idea that this pool of zinc functions as a neuromodulator is attractive. The lack of phenotype in the *Znt3*-null mice suggests either that zinc does not play an important role under normal circumstances, or that there is nearly perfect compensation for the lack of vesicular zinc. The available results suggest that synaptic zinc has the overall effect of inhibiting excitability and thus reducing severity of seizures elicited by kainite [28]. The observation that synaptic zinc contributes to deposition of amyloid plaques is consistent with where plaques accumulate and the observation that zinc chelation ameliorates plaque deposition in an animal model of Alzheimer's [31, 32].

SLC30A4 (human ZNT4; rodent Znt4)

Tissue distribution

Znt4 is widely expressed in rodents [6, 19], but it has been studied primarily in intestinal and mammary epithelial cells [19, 33, 34].

Cellular localization

ZnT4 appears on intracellular vesicles [6, 33, 34, 35]. The endogenous ZnT4 was detected in the Golgi apparatus as well as in the vesicular compartment in rat normal kidney (NRK) cells [10]. Transfection of a Myc-tagged version of ZnT4 into Caco-2 cells revealed expression in an endosomal compartment [33]. Likewise, localization of ZnT4-EGFP in BHK cells was the same as ZnT2; the cells accumulated large amounts zinc (detected with zinquin) in an endosomal compartment and the cells were resistant to zinc toxicity (unpublished observations). In mammary epithelial cells, ZnT4 is reported to be on vesicles, but not those that stain with zinquin [34]. Transfection of mammary epithelial cells with plasmids allowing expression of EGFP-tagged ZnT4 and a secreted protein, human growth hormone revealed co-expression, presumably in secretory granules. However, overexpression of ZnT4 in mammary epithelial cells had no effect on zinquin fluorescence or resistance to zinc toxicity (unpublished observations). It is possible that zinc in secretory granules is not detectable with zinquin because it is tightly bound to various proteins within the vesicles. The cellular localization of ZnT4 may depend on the chaperones present.

Regulation of expression

Zinc has little effect on *Znt4* mRNA levels [19, 34]. However, zinc regulates the intracellular localization of ZnT4. High extracellular zinc concentration induces trafficking of ZnT4 from TGN to the cytoplasmic vesicular compartment in the cultured NRK cells [10].

Functional studies

The *lethal milk (lm)* mouse has a nonsense mutation at position 297 of the 430-amino acid protein [6]. The milk of these mice will not support normal development of pups, but pups can be rescued either by injecting them with zinc or fostering them to the normal dams [7]. In addition, lethal milk mice have few otoliths (calcium carbonate crystals in the semi-circular canal of the inner ear); hence, they have defects in balance and swimming. Older mice develop alopecia and dermatitis [8]. The submaxillary glands of male *lm/lm* mice lack vesicular zinc that is detected with TSQ or zinquin (T. Cole, personal communication). Zinc is secreted from these glands along with nerve growth factor via a classical secretory pathway.

Physiological implications

ZnT4 presumably facilitates zinc entry into secretory vesicles of certain glands (mammary and submaxillary) and thereby allows secretion of zinc by these exocrine glands. The otolith defect may reflect a deficiency of functional carbonic anhydrase, a zinc-requiring enzyme. Endocrine glands apparently use a different family member (see SLC30A5). However, many other cells also express ZnT4 and in those cells it may be associated with different intracellular compartments. Defects in this gene are not responsible for the human inherited disease of zinc metabolism, acrodematidis enteropathica [36].

SLC30A5 (human ZNT5, ZTL1; rodent Znt5)

Tissue distribution

Human *ZNT5* mRNA is widely expressed, but most abundant in pancreatic β cells by Northern blot analysis [9, 37].

Cellular localization

When transfected into HeLa cells under control of a tetracycline-inducible promoter, human ZNT5 was detected in the Golgi complex, but it had no effect on zinquin fluorescence when expression was induced. However, Golgi-enriched vesicles isolated from these cells had greater uptake of ^{65}Zn [9]. In pancreatic β cells ZNT5 was associated with zinc-rich secretory granules that contain insulin, when examined by electron microscopy [9]. Transfection of a Myc-tagged hZTL1 (aa 172–666 of ZNT5) into Caco-2 cells indicated that the protein is expressed on the apical side of cells.

Regulation of expression

The *Znt5* mRNA level increased two-fold when Caco-2 cells were cultured in the medium with progressively increased zinc concentration up to 100 μM $ZnCl_2$ for 7 days [38]. In addition, the expression of the *Znt5* gene was up-regulated in response to balloon catheter injury in rabbit aortas [37].

Functional studies

Loss-of-function studies indicate that *Znt5*-null mice develop hunched backs and osteopenia due to the reduced activity of osteoblasts [37]. Male *Znt5*-null mice die of heart block and sinus bradycardia at 15 weeks of age [37]. Other phenotypes of the *Znt5*-null mice include poor growth, lean body composition, and muscle weakness. Mice heterozygous for *Znt5* are phenotypically normal. The gain-of-function studies do not reveal any zinc phenotype in intact HeLa cells [9]. Expression in yeast results in zinc sensitivity [9]. Expression of mouse ZnT5 in zinc-sensitive BHK cells does not affect zinc sensitivity and does not alter zinquin fluorescence (unpublished observations).

Physiological implications

ZnT5 apparently plays roles in osteoblast maturation in the bone tissue and in maintenance of normal cardiac-conduction in heart. The abundance of ZnT5 in β cells and association with insulin-containing granules suggests that it may be involved in sequestrating zinc in those granules. The low adiposity of *Znt5*-null mice may be the secondary effect of dysfunction of pancreatic β cells. ZnT5 is unusual relative to the other members of this family in having at least 6 and maybe 9 extra membrane-spanning domains. It is similar to a yeast protein, MSC2, which appears regulate zinc functions in the nucleus [39]; thus, it may behave quite differently from other members of the SLC30 family.

SLC30A6 (human ZNT6; rodent Znt6)

Tissue distribution

Mouse *Znt6* mRNA was detected on Northern blots of RNA from liver, kidney, brain and intestine; however, ZnT6 protein was detected by Western blot only in brain and lung [10].

Cellular localization

In cultured rat NRK and human MCF-7 cells, ZnT6 was localized to the TGN and a cytoplasmic vesicular compartment ([10, 11] and unpublished data).

Regulation of expression

No changes in mRNA or protein abundance were detected in NRK and HEK293 cells cultured in the medium containing 100–200 μM $ZnSO_4$ ([10], unpublished data). However, ZnT6 localization is affected by changes in zinc concentration [10]. ZnT6 is localized predominantly in the TGN in NRK cells in the normal cultured condition, but it relocates to the cytoplasmic vesicular compartment in response to the high extracellular zinc concentration [10].

Functional studies

No functional role for ZnT6 in mammalian cells has been described. Expression of *Znt6* in yeast inhibits growth in a zinc-dependent manner in strains bearing mutations in the *zrt1*, *zrt3*, and *msc2* genes [10]. Site-directed mutagenesis analysis of ZnT6 indicates that three serine residues in the His-rich region (aa 164, 168, and 170) and two histidine residues at the C-terminal end of ZnT6 (aa 259 and 300) are critical for its ability to inhibit growth of yeast (unpublished data).

Physiological implications

The similarity with ZnT2, 3, and 4 and its localization in the TGN suggest that ZnT6 may be important for transport of zinc into that compartment.

SLC30A7 (human ZNT7, ZNTL2; rodent Znt7)

Tissue distribution

Znt7 mRNA was detected in a variety of mouse tissues including liver, kidney, spleen, heart, brain, small intestine, and lung by Northern blot analysis; however, ZnT7 protein was only detected in small intestine and lung by Western blot analysis [11].

Cellular localization

ZnT7 was localized to the Golgi apparatus and a cytoplasmic vesicular compartment, which is different from those of ZnT2, ZnT4, and ZnT6, in the cultured rat small intestinal epithelia and human lung fibroblasts. The Myc-tagged ZnT7 was localized in the same compartments in the transfected NRK and Chinese hamster ovary cells [11].

Regulation of expression

No changes in mRNA or protein abundance were detected in HEK293 cells and human B-lymphocytes cultured in the medium containing 30–75 μM $ZnSO_4$ (unpublished data). The intracellular localization of ZnT7 is unaffected by the changes of extracellular zinc concentration (unpublished data).

Functional studies

Stable expression of ZnT7 in CHO cells results in accumulation of zinc in the Golgi apparatus as detected by zinquin [11].

Physiological implications

Because ZnT7 protein is abundantly expressed in the small intestine it may play an important role in zinc absorption by the gut.

SLC30A8 (human ZNT8; rodent Znt8)

Tissue distribution

Human *ZNT8* mRNA was only detected in the brain and liver tissues (unpublished data).

Cellular localization

Nothing reported.

Regulation of expression

Nothing reported.

Functional studies

Nothing reported.

Physiological implications

Unknown.

SLC30A9 (human ZNT9; rodent Znt9)

There are no publications related to this orphan member of the SLC30 family.

References

1. Palmiter RD, Findley SD (1995) Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. EMBO J 14:639–649
2. Palmiter RD, Cole TB, Findley SD (1996) ZnT-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. EMBO J 15:1784–1791
3. Kamizono A, Nishizawa M, Teranishi Y, Murata K, Kimura A (1989) Identification of a gene conferring resistance to zinc and cadmium ion in the yeast *Saccharomyces cerevisiae*. Mol Gen Genet 219:161–167
4. Conklin DS, Culbertson MR, Kung C (1994) Interactions between gene products involved in divalent cation transport in *Saccharomyces cerevisiae*. Mol Gen Genet 244:303–311
5. Palmiter RD, Cole TB, Quaife CJ, Findley SD (1996) ZnT-3, a putative transporter of zinc into synaptic vesicles. Proc Natl Acad Sci USA 93:14934–14939
6. Huang, L, Gitschier J (1997) A novel gene involved in zinc transport is deficient in the lethal milk mouse. Nat Genet 17:292–297
7. Piletz JE, Ganschow RE (1978) Zinc deficiency in murine milk underlies expression of the *lethal milk (lm)* mutation. Science 199:181–183
8. Erway LC, Grider A Jr (1994) Zinc metabolism in lethal-milk mice: otolith, lactation, and aging effects. J Hered 75:480–484
9. Kambe T, Narita H, Yamaguchi-Iwai Y, Hirose J, Amano T, Sugiura N, Sasaki R, Iwanaga T, Nagao M (2002) Cloning and characterization of a novel mammalian zinc transporter, zinc transporter 5, abundantly expressed in pancreatic beta cells. J Biol Chem 277:19049–19055
10. Huang L, Kirschke CP, Gitschier J (2002) Functional characterization of a novel mammalian zinc transporter, ZnT6. J Biol Chem 277:26389–26395
11. Kirschke CP, Huang L (2002) ZnT7, A novel mammalian zinc transporter, accumulates zinc in the Golgi apparatus. J Biol Chem 278:4096–4102
12. Gaither LA, Eide DJ (2001) Eukaryotic zinc transporters and their regulation. Biometals 14:251–270
13. Nies DH, Silver S (1995) Ion efflux systems involved in bacterial metal resistances. J Ind Microbiol 14:186–199
14. Paulsen IT, Saier MH Jr (1997) A novel family of ubiquitous heavy metal ion transport proteins. J Membr Biol 156:99–103
15. Guffanti AA, Wei Y, Rood SV, Krulwich TA (2002) An antiport mechanism for a member of the cation diffusion facilitator family: divalent cations efflux in exchange for K^+ and H^+. Mol Microbiol 45:145–153
16. MacDiarmid CW, Milanick MA, Eide DJ (2002) Biochemical properties of vacuolar zinc transport systems of *Saccaromyces cerevisiae*. J Biol Chem 277:39187–39194
17. Gunshin J, MacKenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482–488
18. McMahon RJ, Cousins RJ (1998) Regulation of the zinc transporter ZnT-1 by dietary zinc. Proc Natl Acad Sci USA 95:4841–4846
19. Liuzzi JP, Blanchard RK, Cousins RJ (2001) Differential regulation of zinc transporter 1, 2 and 4 mRNA expression by dietary zinc in rats. J Nutr 131:46–52
20. Sekler I, Moran A, Hershfinkel M, Dori A, Margulis A, Birenzweig N, Nitzan Y, Silverman WF (2002) Distribution of the zinc transporter ZnT-1 in comparison with chelatable zinc in the mouse brain. J Comp Neurol 447:201–209
21. Langmade SJ, Ravindra R, Daniels PJ, Andrews GK (2000) The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene. J Biol Chem 275:34803–34809
22. Tsuda M, Imaizzumi K, Katayama T, Kitagawa K, Wanaka A, Tohyama M, Takagi T (1997) Expression of the zinc transporter gene, ZnT-1, is induced after transient forebrain ischemia in the gerbil. J Neurosci 17:6678–6684
23. Kobayashi T, Beuchat M-H, Lindsay SF, Palmiter RD, Sakuraba H, Parton RG, Gruenberg J (1999) Late endosomal membranes rich in lysobisphosphatidic acid regulate cholesterol transport. Nat Cell Biol 2:113–118
24. Wenzel HJ, Cole TB, Born DE, Schwartzkroin PA, Palmiter RD (1997) Ultrastructural localization of zinc transporter 3 (ZnT-3) to synaptic vesicle membranes within mossy fiber boutons in the hippocampus of mouse and monkey. Proc Natl Acad Sci USA 94:12676–12681
25. Cole TB, Wenzel HJ, Kafer KE, Schwartzkroin PA, Palmiter RD (1999) Elimination of zinc from synaptic vesicles in intact mouse brain by disruption of the *ZnT3* gene. Proc Natl Acad Sci USA 96:1716–1721
26. Kantheti P, Qiao X, Diaz ME, Peden AA, Meyer GE, Carskadon SL, Kapfhamer D, Sufalko D, Robinson MS, Noebels JL, Burmeister M (1998) Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes and synaptic vesicles. Neuron 21:111–122
27. Cole TB, Martyanova A, Palmiter RD (2001) Removing zinc from synaptic vesicles does not impair learning, memory, or sensorimotor functions in the mouse. Brain Res 891:253–265
28. Cole TB, Robbins CA, Wenzel HJ, Schwartzkroin PA, Palmiter RD (2000) Seizures and neuronal damage in mice lacking vesicular zinc. Epilepsy Res 39:153–169
29. Lee J-Y, Cole TB, Palmiter RD, Koh J-Y (2000) Accumulation of zinc in degenerating hippocampal neurons on ZnT3-null mice after seizures: evidence against synaptic vesicle origin. J Neurosci 20:RC79 (1–5)
30. Lopantsev V, Wenzel HJ, Cole TB, Palmiter RD, Schwartzkroin PA (2003) Lack of vesicular zinc in mossy fibers does not affect synaptic excitability of CA3 pyramidal cells in zinc transporter 3 knockout mice. Neuroscience 116:237–248
31. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, Barnham KJ, Volitakis I, Fraser FW, Kim Y, Huang X, Goldstein LE, Moir RD, Lim JT, Beyreuther K, Zheng H, Tanzi RE, Masters CL, Bush AI (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 30:665–676
32. Lee J-Y, Cole TB, Palmiter RD, Suh SW, Koh J-Y (2002) Contribution by synaptic zinc to the gender-disparate plaque formation in human Swedish mutant APP transgenic mice. Proc Natl Acad Sci USA 99:7705–7710
33. Murgia C, Vespignani I, Cerase J, Nobili F, Perozzi G (1999) Cloning, expression and vesicular localization of zinc transporter Dri27/ZnT4 in intestinal tissue and cells. Am J Physiol 277:G1231–1239
34. Michalczyk AA, Allen J, Blomeley RC, Ackland ML (2002) Constitutive expression of hZnT4 zinc transporter in human breast epithelial cells. Biochem J 364:105–113
35. Kelleher SL, Lonnerdal B (2002) Zinc transporters in the rat mammary gland respond to marginal zinc and vitamin A intakes during lactation. J Nutr 132:3280–85
36. Bleck O, Ashton GH, Mallipeddi R, South AP, Wittock NV, McLean WH, Atherton DJ, McGrath JA (2001) Genomic localization, organization and amplification of the human zinc transporter protein gene, ZNT4, and exclusion as a candidate in different variants of acrodermatidis enteropathica. Arch Dermatol Res 293:392–396
37. Inoue K, Matsuda K, Itoh M, Kawaguchi H, Tomoike H, Aoyagi T, Nagai R, Hori M, Nakamura Y, Tanaka T (2002) Osteopenia and male-specific sudden cardiac death in mice lacking a zinc transporter gene, Znt5. Hum Mol Genet 11:1775–84
38. Cragg RA, Christie GR, Phillips SR, Russi RM, Kury S, Mathers JC, Taylor PM, Ford D (2002) A novel zinc-regulated human zinc transporter, hZTL1, is localized to the enterocyte apical membrane. J Biol Chem 277:22789–97
39. Li L, Kaplan J (2000) The yeast gene MSC2, a member of the cation diffusion facilitator family, affects cellular distribution of zinc. J Biol Chem 275:5036–5043